1.INTRODUCTION

While food has long been used to improve health, our knowledge of the relationship between food components and health is now being used to improve food. All food is functional, in that it provides energy and nutrients necessary for survival. But the term "FUNCTIONAL FOOD" includes conventional foods; fortified, enriched or enhanced foods; and dietary supplements. These substances provide essential nutrients often beyond quantities necessary for normal maintenance, growth, and development, and/or other biologically active components that impart health benefits or desirable physiological effects. Functional foods can take many forms. Some may be conventional foods with bioactive components that can now be identified and linked to positive health outcomes. Some may be fortified or enhanced foods, specifically created to reduce disease risk for a certain group of people. Consumers can already select from a wide spectrum of foods that contain functional components either inherently (e.g., soy protein, cranberries) or via fortification (e.g., folate-fortified foods). Health benefits may result from increasing the consumption of substances already part of an individual's diet or from adding new substances to an individual's diet.

1.1 ENCOURAGING THE DEVELOPMENT OF FUNCTIONAL FOODS

As research provides clear evidence of relationships between dietary components and health benefits, the challenge has just begun. Scientific, regulatory, and business frameworks must be in place to evaluate the data for efficacy and safety, ensure effective regulatory oversight, communicate the findings to consumers, and provide incentives that encourage research and development of these novel food products. There should be modifications to the existing efficacy and safety evaluation process to ensure a sound scientific underpinning for each proposed functional food, while providing clear information to consumers, corresponding to the improvements in the regulatory oversight of new functional components. These changes must be implemented to protect consumer confidence in the safety of the food supply and to encourage the food industry to invest in the development of new functional foods.

The functional foods currently available represent only a fraction of the potential opportunities for consumers to manage health through diet. Traditional definitions and arbitrary distinctions between food and medicine should not prevent consumer access to knowledge about the benefits of incorporating functional foods into their diets. Likewise, the framework for providing strong regulatory oversight should not present unnecessary barriers to the development and marketing of functional foods. Where existing terminology and

regulatory frameworks are inadequate to address the full scope of benefits and opportunities for functional foods, the terminology and the frameworks must be modified. Developing a new functional food is an expensive process. Food companies have traditionally funded research for new food product formulations but for functional foods, the stakes are higher—for both food companies and consumers. Government investment in basic and applied research will promote the development of functional foods, but additional incentives are needed to reward private companies that pioneer new health claims. The research required for a functional food to meet scientific standards for efficacy and safety is a substantial investment, but currently the return on that investment is not exclusive to the company that conducted the research and developed the initial regulatory petition. As soon as the health claim is adequately documented, competing companies can use the claim. Incentives, such as a period of exclusivity or tax incentives, would encourage food companies to pursue functional food development by ensuring a profitable return on successful products (IFIC, 2004).

In recent years, these changes and developments have led to newer products in the beverages sector. One such major functional requirement is milk alternatives to answer problems of cow milk allergy, lactose intolerance, calorie concern and prevalence of hypercholesterolemia (Valencia-Flores *et al.*, 2013).

As a result, the consumption of plant based milk has been increased due to absence of cholesterol and lactose, making it suitable for a group of population suffering from lactose intolerance and heart disease and in general for all. The market for plant based milk alternatives is projected to grow at a CAGR of 15 % from 2013 to 2018 (Markets and Markets, 2013) and is expected to reach a value of $14 billion. In recent years, plant sources (cereals and legumes) are accepted as functional food and nutraceuticals due to presence of health promoting components such as dietary fibres, minerals, vitamins and antioxidants (Das *et al.*, 2012).

All plant-based milks are preferred over cow's milk by consumers who are lactose intolerant or are allergic to milk proteins because of their common benefits of being lactose free, cholesterol free and low in calories. Plant-based milk alternatives are prepared by disintegration of plant material, which makes the particle composition and size as non-uniform. The size of particles and the stability of the final product depend on the nature of the raw material, the method used for disintegration and storage conditions (Cruz *et al*., 2007).

Few researches in preparing milk alternates other than soy milk includes peanut milk, rice milk, oat milk, sesame milk, coconut milk, almond milk, hemp milk, hazelnut milk, tiger nut, lupin milk and quinoa milk etc.

Plant-based milk alternatives with technological interventions applied so far for their quality improvement; and future research efforts that can be carried out in development of quality plant based milk alternatives (Sethi *et al.*, 2016).

1.2 PLANT-BASED / NON-DAIRY MILK ALTERNATIVES

Plant-based milk alternatives are fluids that results from breakdown (size reduction) of plant material (cereals, pseudo-cereals, legumes oilseeds, nuts) extracted in water and further homogenisation of such fluids, results in particle size distribution in range of 5–20 lm which imitates cow's milk in appearance and consistency. Although, there is no stated definition and classification of these plant based milk alternatives in literature, a general classification of the plant based/vegetable milk alternatives into five categories is attempted, which is as follows:

(a) Cereal based: Oat milk, Rice milk, Corn milk, Spelt milk.

(b) Legume based: Soy milk, Peanut milk, Lupin milk, Cowpea milk.

(c) Nut based: Almond milk, Coconut milk, Hazelnut milk, Pistachio milk, Walnut milk.

(d) Seed based: Sesame milk, Flax milk, Hemp milk, Sunflower milk.

(e) Pseudo-cereal based: Quinoa milk, Teff milk, Amaranth milk.

In this regard, chick peas and cowpeas will be used in my study for preparation of non-dairy, healthy, affordable and nutritious plant based milk alternative.

1.3 CHICKPEAS (*Cicer arietinum L.*)

1.3.1 CULTIVATION

Chickpeas (*Cicer arietinum* L.), commonly known as garbanzo beans, are an old world pulse (i.e., edible seeds) in the legume family (USDA, 2016) and have traditionally been incorporated into many culinary creations because of their nut-like flavor and versatile sensory applications in food (Deosthale, 1988). The origin of the chickpeas is thought to have been Levant and ancient Egypt (Davidson, 1999), which is logical since the plant prefers temperate and semiarid regions.

India is the world's leading producer of chickpeas. Worldwide over 14.2 metric tons of chickpeas were harvested in 2014 according to the Food and Agriculture Organization (FAO) of the United Nations (FAOSTAT, 2013). Two main varieties of chickpeas exist: the light seeded Kabuli type and the smaller dark Desi type (Huntrods, 2013).

1.3.2 NUTRITIVE VALUE

Pulses are unique in comparison to other plant foods in that they contain higher proportions of protein (17%–30% by dry weight). The main proteins found in chickpeas, similar to other legumes, are albumins and globulins. Smaller amounts of glutelins and prolamines are also present (Saharan, 1994). Starch is the major storage carbohydrate followed by dietary fibre, oligosaccharides and simple sugars such as glucose and sucrose. Although lipids are present in low amounts, chickpea is rich in nutritionally important unsaturated fatty acids such as linoleic and oleic acids. β-Sitosterol, campesterol and stigmasterol are important sterols present in chickpea oil. Ca, Mg, P and, especially, K are also present in chickpea seeds. Chickpea is a good source of important vitamins such as riboflavin, niacin, thiamin, folate and the vitamin A precursor β-carotene. As with other pulses, chickpea seeds also contain anti-nutritional factors which can be reduced or eliminated by different cooking techniques.

1.3.3 HEALTH BENEFITS

Chickpea has several potential health benefits, and, in combination with other pulses and cereals, it could have beneficial effects on some of the important human diseases such as C.V.D., type-2 diabetes, digestive diseases and some cancers. Overall, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits (Jukanti *et al.*, 2012).

1.4 COWPEA (*Vigna unguiculata*)

1.4.1 CULTIVATION

The cowpea (*Vigna unguiculata*) is an annual herbaceous legume from the genus *Vigna*. Due to its tolerance for sandy soil and low rainfall it is an important crop in the semi-arid regions across Africa and other countries. Four subspecies of cowpea are recognised, of which three are cultivated. There is a high level of morphological diversity found within the species with large variations in the size, shape and structure of the plant. Cowpeas can be erect, semi erect

(trailing) or climbing. The crop is mainly grown for its seeds, which are extremely high in protein, although the leaves and immature seed pods can also be consumed.

Cultivated cowpeas are known by the common names black-eyed pea, southern pea, yardlong bean, catjang, and crowder pea. They were domesticated in Africa and are one of the oldest crops to be farmed. A second domestication event probably occurred in Asia, before they spread into Europe and the Americas. The seeds are usually cooked and made into stews and curries, or ground into flour or paste (Egbadzor, 2014).

1.4.2 NUTRITIVE VALUE

Cowpeas seeds provide a rich source of proteins and calories, as well as minerals and vitamins (Goncalves, 2016). A seed can consist of 25% protein and has very low fat content (Rangel, 2003). Cowpea starch is digested more slowly than the starch from cereals, which is more beneficial to human health (Goncalves, 2016). The grain is a rich source of folic acid, an important vitamin that helps prevent neural tube defects in unborn babies (Witthoft, 2016).

The cowpea has often been referred to as "poor man's meat" due to the high levels of protein found in the seeds and leaves (Hamid, 2016). However, it does contain some anti-nutritional elements, notable phytic acid and protease inhibitors, which reduces the nutritional value of the crop (Goncalves, 2016).

1.4.3 HEALTH BENEFITS

Cowpeas health benefits includes improving digestion, supporting heart health, detoxifying the body, treating insomnia, managing diabetes, and supporting blood circulation. It is also good for weight loss, removes free radicals and controls blood cholesterol (Goncalves, 2016).

Now-a-days, probiotic bacteria are available in a variety of food products, dietary supplements and drugs. Most probiotic products are dairy based since ancient times. Dairy is a very nutritious substrate which satisfies the nutritional requirements of fastidious LAB. But, expanding trend of vegan lifestyles, the issues of lactose intolerance and demand for low-fat and low cholesterol foods have created a growing demand for new taste with non-dairy matrics and trend of vegetarianism are increasing (Ranadheera *et al*., 2010). This leads to increased demand for non-dairy probiotic foods, such as coconut milk, fruit drinks, nutrition bars, soy products/ legume-based and cereal-based products.

1.5 PROBIOTICS

Schrezenmeir & Vrese (2001) defined probiotics as "a viable microbial food supplement which beneficially influences the health of the host". The composition of the gut microflora is constant but can be affected by some factors such as age, diet, environment, stress and medication. The majority of probiotic microorganisms belong to the genera *Lactobacillus* and *Bifidobacterium*. *Lactobacilli* and *Bifidobacteria* are gram-positive lactic acid-producing bacteria that constitute a major part of the normal intestinal microflora in animals and humans (Vrese & Schrezenmeir, 2008).

Lactobacilli are non-spore forming rod-shaped bacteria. They have complex nutritional requirements and are strictly fermentative, aero-tolerant or anaerobic, and aciduric or acidophilic. *Lactobacilli* are found in a variety of habitats where rich, carbohydrate-containing substrates are available, such as on human and animal mucosal membranes, on plants or material of plant origin, on sewage, and on fermented milk products, fermenting or spoiling food (Vrese & Schrezenmeir,2008).

Bifidobacteria comprises a major part of the normal intestinal microflora in humans throughout life. The number of *Bifidobacteria* in the colon of adults is 10^{10}-10^{11} cfu/gram, but this number decreases with age. Most of the *Bifidobacterial species* are strictly anaerobic, non-motile, and non-sporulating rods with a varying morphological appearance (Vrese & Schrezenmeir, 2008).

1.5.1 FOOD SOURCES OF PROBIOTICS

The most common sources of probiotics are yogurt, cultured buttermilk, and cheese. Cultured buttermilk is made with bacteria that produce lactic acid, which makes the product sour. Some cultured cheese use bacteria to finish the maturation of the cheese. Another fermented dairy product is *Kefir*. However, many studies show that probiotics strains are also found in non-dairy fermented substrates (Vrese & Schrezenmeir, 2008).

Some of the non-dairy probiotic products are cereal, legume, cabbage, maize, pearl millet, vegetable and sorghum (Roberforid, 2007).

1.5.2 HEALTH BENEFITS OF PROBIOTICS

Probiotics may play a beneficial role in several medical conditions, including diarrhoea, gastro-enteritis, irritable bowel syndrome, inflammatory bowel disease, cancer, depressed immune function, infant allergies, failure-to-thrive, hyper-lipidemia, hepatic diseases, *Helicobacter pylori* infections, and others, all of which were suggested by certain research studies to improve with the use of probiotics (Brown & Valiere, 2004).

1.6 PREBIOTICS

Prebiotics are "indigestible fermented food substrates that selectively stimulate the growth, composition, and activity of microflora in gastrointestinal tract and thus improve hosts' health and well-being" (Roberfroid, 2000). Fructo-oligosaccharides, inulin, oligo-fructose, lactulose, and galacto-oligosaccharides have been identified as prebiotics due to characteristics such as resistance to gastric acidity and hydrolysis by mammalian enzymes and they are fermented by gastro-intestinal microflora to further selectively stimulate the growth and activity of beneficial microorganisms. The number of new compounds which have gut resistant properties and selective fermentability by intestinal microorganisms are identified and developed as prebiotics (Ooi & Liong, 2010 ; Gibson & Fuller, 2000). These include oligosaccharides (isomalto-oligosaccharides, lacto-sucrose, xylo-oligosaccharides and gluco-oligosaccharides), sugar alcohols, and polysaccharides (Cummings, 2001).

Fermentation of oligo-fructose in the colon is due to the presence of intestinal microflora, which confers beneficial effects to humans, which include increasing the numbers of probiotics in the colon, increasing calcium absorption, increasing faecal weight, shortening gastrointestinal transit time, and possibly, lowering blood lipid levels.

The prebiotics chosen for the current study are chicory root powder, raw banana powder and almonds.

(a) CHICORY ROOT POWDER

Chicory (*Cichorium intibus* L.) is a biennial plant in the *Asteraceia* family that is also known as succory, hendibeh, blue daisy, blue dandelion, blue weed and coffeeweed. The root stores the carbohydrate energy known as inulin (Moser *et al*., 2014). As a dietary fiber, chicory root fiber remains unabsorbed and undigested until it reaches the large intestine. Microbiota in the large intestine, particularly *Bifidobacteria* and *Lactobacilli*, use chicory root fiber as a nutrient. The breakdown products of this saccharolytic

prebiotic fermentation are short chain fatty acids and other metabolites. Chicory root fiber has additional texturizing and sensorial properties that allow for fiber enrichment as well as for sugar or fat replacement in a large variety of product applications including e.g. bakery foods, cereals, dairy products and alternatives, confectionery, beverages, spreads, soups and sauces, dressings, ice cream, meat and vegetable products, snack, granola, and nutrition bars as well as infant and special purpose nutrition (Moser & Wouters, 2014).

(b) RAW BANANA POWDER

Banana is one of the most consumed fruits in tropical and subtropical regions of Southeast Asia, belongs to family *Musaceae* and class *Liliopsida*. Banana is the fourth largest fruit crop of the world.

225g (7.94 oz.) grams of Bananas (raw) contains 51.39 grams of carbohydrates which is 17% of your recommended daily carbohydrate intake and it contains 5.85 grams of dietary fiber according to the Food and Drug Administration guidelines for a 2000 calorie diet.

(c) ALMONDS

The almond (*Prunus dulcis*, syn. *Prunus amygdalus*) is a species of tree native to Mediterranean climate regions of the Middle East, from Syria and Turkey to India and Pakistan, although it has been introduced elsewhere.

Almond is also the name of the edible and widely cultivated seed of this tree. Within the genus *Prunus*, it is classified with the peach in the subgenus *Amygdalus*, distinguished from the other subgenera by corrugations on the shell (endocarp) surrounding the seed.

The fruit of the almond is a drupe, consisting of an outer hull and a hard shell with the seed, which is not a true nut, inside. Shelling almonds refers to removing the shell to reveal the seed. Almonds are sold shelled or unshelled. Blanched almonds are shelled almonds that have been treated with hot water to soften the seed-coat, which is then removed to reveal the white embryo.

The almond is a nutritionally dense food (see chart pictured right) and a 100 gram amount is a rich source (>20% of the Daily value, DV) of the B vitamins riboflavin and niacin, vitamin E, and the essential minerals calcium, iron, magnesium, manganese, phosphorus, and zinc. The same amount is also a good source (10–19% DV) of the B vitamins

thiamine, vitamin B_6, and folate; choline; and the essential mineral potassium. They are also rich in dietary fiber, monounsaturated fats, and polyunsaturated fats, fats which potentially may lower LDL cholesterol (Berryman *et al.,* 2011). 100 grams of almonds contains 12.2 grams of dietary fiber (USDA Nutrient Database).

1.7 SYMBIOTIC EFFECTS OF PREBIOTICS AND PROBIOTICS

The combination of prebiotic and probiotic has synergistic effects because in addition to promoting growth of existing strains of beneficial bacteria in the colon, symbiotics also act to improve the survival, implantation, and growth of newly added probiotic strains.

2. REVIEW OF LITERATURE

2.1 NEED OF NON-DAIRY PROBIOTICS

Asian diets are relatively low in meat and dairy foods, and plant-based foods contribute the core of the daily intake. Besides dietary habits, lactose intolerance discourages many Asian people from consuming milk. The average per capita dairy consumption (including fluid milk, butter, cheese, NFD, and WMP) in milk equivalent in the last decade for the major dairy markets was 10.2 kg in China, 71.8 kg in India, 7.8 kg in Indonesia, 97.6 kg in Japan, 67.8 kg in Malaysia, 24 kg in the Philippines, 80 kg in South Korea, 28.7 in Thailand, and 8.6 kg in Vietnam. This contrasts with per capita consumption of 330 kg in the EU-15, 310 kg per capita in Australia, and 251 kg per capita in the United States (Dong, 2006). Therefore, considering the above mentioned facts cereals, fruits and vegetable may be potential substrates, where the healthy probiotic bacteria will make their mark, both in the developing and the developed countries.

2.2 FUNCTIONALITY OF PROBIOTICS

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children. However, some health benefits, e.g., immune modulation, may be achieved even with dead bacteria (Kalliomaki *et al.*, 2001).

Bacterial cultures, yogurt starter cultures as well as some probiotic cultures are known to improve the lactose digestion in lactose mal-digestors. Subjects suffering from lactose intolerance have a very low concentration of the lactose-cleaving enzyme *β-galactosidase*, and bacteria in fermented or unfermented food products release their *β-galactosidase* in the small intestine, where it supports lactose digestion (Li *et al*., 2012).

Respiratory effects of probiotics in animal models have included attenuating allergic airway responses and protecting against respiratory pathogens. Dendritic cells appear central to directing the beneficial immune response to probiotic bacteria and in translating microbial signals from the innate to the adaptive immune system, whereas regulatory T cells are emerging as potentially key effectors of probiotic mediated responses, particularly in the reduction of allergic inflammation. Despite progress in basic research, clinical trials of probiotics in allergy/asthma and respiratory infection have been highly variable at best, leading to an undermining of confidence in this potential therapeutic strategy (Forsythe, 2011).

Probiotics have also been used successfully in the management of atopic eczema in infants. Furthermore, *Lactobacillus rhamnosus* GG proved to be effective in the prevention of early atopic disease in children at high risk. The *Lactobacillus rhamnosus* GG product when given prenatally to mothers and post-natally for 6 months to the mothers or to their infants directly, reduced the frequency of atopic eczema in the probiotic group to half that of the placebo group at the age of 2 years. The preventive effect was reconfirmed at the age of 4 years (Delcenserie *et al*., 2010).

Several probiotic strains, especially *Lactobacillus rhamnosus* have been shown to prevent or alleviate infantile diarrhoea, caused mainly by rotavirus. It is also well-established that some probiotic strains can both prevent and shorten antibiotic-associated disorders. (Fuller *et al*., 2008).

Two probiotic strains have been developed with a particular focus on their enhancing effects on immune responses: *Bifidobacterium lactis* and *Lactobacillus rhamnosus*. Both strains have been demonstrated in several studies to enhance natural immune function in healthy people (Fuller *et al.*, 2008).

Several probiotic strains have been shown to inhibit *Helicobacter pylori* in vitro, which has been associated with gastritis, stomach carcinoma, gastric ulcer, and lymphomas. Human

studies confirmed this inhibitory effect on *Helicobacter pylori*, which seems to be independent of the viability of the bacteria (Hamilton-Miller *et al*., 2003).

Many human studies have evaluated the effects of culture-containing dairy products or probiotic bacteria on cholesterol levels with equivocal results. A fermented milk containing *Enterococcus faecium* and *Streptococcus thermophilus* was reported to produce a small but significant decrease in total and LDL-cholesterol in patients with primary hypercholesterolemia (Pereira *et al*., 2002).

2.3 CURRENT RECENT TRENDS IN PROBIOTICS

Waters *et al.,* (2015) consolidated the findings of various research workers in determining the role of LAB in the fermentation of cereals used for the preparation of beverages.

Sorghum flour based yoghurt like product 'sorghurt' was prepared recently and it had a viable count of >8 log10 cfu/mL of the product, which is higher than the minimum desirable count of 10^6 cfu/g, with acceptable sensory scores (Sanni *et al.*, 2013).

Kalui *et al*., (2010) reviewed the probiotic potential of spontaneously fermented cereal based foods. As an alternate to dairy based probiotic foods, single and mixed cereals (barley and malt) based probiotic beverages containing *L. plantarum* and *L. acidophilus* in the range of 7.9 and 8.5 log cfu/mL have been developed (Rathore *et al.*, 2012).

Rakin *et al.,* (2007) enriched beetroot and carrot juices with the brewer's yeast autolysate before lactic acid fermentation with *Lb. acidophilus*. The addition of the autolysate favorably affected the increase of the number of lactic acid bacteria during the fermentation (Aeschlimann and Stocar, 1990), reduction of the time of fermentation and enrichment of the vegetable juices with amino acids, vitamins, minerals and antioxidants (Chae, Joo, and In, 2001). The use of spent brewer's yeast from the brewery was important for the economic optimization of the fermentation. A mixture of beetroot and carrot juices has optimum proportions of pigments, vitamins and minerals (Rakin *et al.*, 2007).

According to Sheehan *et al.,* (2007), when adding *Lactobacillus* and *Bifidobacterium* orange, pineapple and cranberry juice, extensive differences regarding their acid resistance were observed. All of the strains screened survived for longer in orange and pineapple juice compared to cranberry. *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei* display a great robustness surviving at levels above 7.0 log cfu/ml in orange juice

and above 6.0 log cfu/ ml in pineapple juice for at least 12 weeks. However, after thermal pasteurization at 76°C for 30 s and 90°C for 1 min in addition to a high-pressure treatment of 400MPa for 5 min, *Lb. casei*, *Lb. rhamnosus* and *Lb. paracasei* were not capable of withstanding the treatments required to achieve a stable juice at levels >6.0 log cfu/ml (Sheehan *et al*., 2007).

Angelov *et al.* (2006) produced a symbiotic functional drink from the oats by combining a probiotic starter culture and whole-grain oat substrate. The oats and barley are the cereals with highest content of β-glucan, recognized as the main functional component of the cereal fibers. Studies have indicated the hypocholesterolemic effect of this compound, leading to 20–30% reduction of LDL-cholesterol, and to an expected overall effect of reduced cardiovascular disease risk (Stark and Madar, 1994). The substrate was fermented with *Lb. plantarum* B28. The levels of starter culture concentration, oat flour and sucrose content were established for completing a controlled fermentation for 8 h. The addition of aspartame, sodium cyclamate, saccharine and Huxol (12% cyclamate and 1.2% saccharine) had no effect on the dynamics of the fermentation process and on the viability of the starter culture during the product storage. The β-glucan content in the drink of 0.31–0.36% remained unchanged throughout fermentation and storage of the drink. The viable cell counts reached at the end of the process were about 7.5 x 10^{10} cfu/mL. The shelf life of the oat drink was estimated to 21 days under refrigerated storage.

Vita Biosa is a mixture of aromatic herbs and other plants, which are fermented by a combination of lactic acid and yeast cultures. This beverage contains no sugar and can produce carbon dioxide. The bacteria are selected based on the criterion of their providing the microflora of the intestines with the best possible conditions. During the fermentation, the lactic acid formed produces a low pH value of about 3.5. The low pH prevents the development of harmful bacteria in the finished product. *Vita Biosa* also contains a high number of antioxidants. The reason for this beverage becoming so popular is its ability to quickly restore the natural balance in the digestive system. The liquid restricts harmful bacteria and thus gives the beneficial bacteria a better possibility to multiply and create healthy intestinal flora. *Vita Biosa* is manufactured in Denmark (Kalui *et al*., 2010).

Yoon *et al.*, (2006) also developed a probiotic cabbage juice using lactic acid bacteria. Cabbage juice was inoculated with a 24 h-old lactic culture and incubated at 30°C. The cultures (*Lb. plantarum C3, Lb. casei A4 and Lb. delbrueckii D7*) grew well on cabbage juice

and reached about 1 x 10^9 cfu/mL after 48 h of the fermentation. *Lb. casei* produced a lower amount of titratable acidity, expressed as lactic acid, than *Lb. delbrueckii,* or *Lb. plantarum*. After 4 weeks of the cold storage at 4°C, the viable cell counts of *Lb. plantarum* and *Lb. delbrueckii* were 4.1 x 10^7 and 4.5 x 10^5 CFU/mL, respectively. *Lb. casei* did not survive at low pH and lost cell viability completely after 2 weeks of the cold storage. The fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Soybean has received attention from the researchers due to its protein quality. Soymilk is suitable for the growth of the lactic acid bacteria, especially *Bifidobacteria* (Chou and Hou, 2000). Several studies have mentioned the production and use of the fermented soymilk drinks as probiotic, mainly soybean yogurt, which further can be supplemented with oligofructose and inulin (Fuchs *et al.*, 2005).

Yoon *et al*., (2004) determined the suitability of the tomato juice as a raw material for the production of probiotic juice by *Lb. acidophilus LA39*, *Lb. plantarum C3*, *L. casei A4* and *Lb. delbrueckii D7*. The tomato juice was inoculated with a 24 h-old culture and incubated at 30°C. The lactic acid cultures reduced the pH to 4.1 and the viable cell counts reached nearly (1.0– 9.0) x 10^9 cfu/mL after 72 h fermentation. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 cfu/mL after 4 weeks of cold storage at 4°C. Yoon *et al.,* (2005) also evaluated the potential of red beets as the substrate for the production of probiotic beet juice by the above four species of lactic acid bacteria. All the lactic cultures were capable of rapidly utilizing the beet juice for the cell synthesis and lactic acid production. *Lb. acidophilus* and *Lb. plantarum* produced higher amount of lactic acid than other cultures and reduced the pH of the fermented beet juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30°C. Although the lactic cultures in fermented beet juice gradually lost their viability during the cold storage, the viable cell counts of these bacteria, except for *Lb. acidophilus*, in the fermented beet juice still remained at 10^6–10^8 cfu/ mL after 4 weeks of cold storage at 4°C.

Malted barley is added to enhance the liquefaction of the product and *Lb. plantarum* 299v carries out the fermentation. The final product contains 1 x 10^{12} colony-forming units (CFU) of *L. plantarum*. This formula is used as the active ingredient in the food product in which 5% of the oatmeal gruel is mixed with a fruit drink. The consumer product contains 5 x 10^{10} CFU/mL (Molin, 2001).

2.4 HEALTH BENEFITS OF SYNBIOTICS

Kleessen *et al*., (2017) studied the effects of inulin and lactose on fecal microflora, microbial activity and bowel habit in elderly constipated persons and concluded that Inulin increased *bifidobacteria* significantly, but decreased *enterococci* in number and *entererobacteria* in frequency. For individuals consuming lactose, increase in fecal counts of *enterococci* and a decrease in *lactobacilli* and *clostridia* was detected, although the total bacterial count remained unchanged. In this study, Inulin showed a better laxative effect than lactose and reduced functional constipation with mild discomfort.

Mishra *et al.,* (2015) studied probiotics as potential antioxidants and found out that consumption of probiotics alone or foods supplemented with probiotics may reduce oxidative damage, free radical scavenging rate, and modification in activity of crucial anti-oxidative enzymes in human cells. The methods used are modifications of those used for the determination of the anti-oxidative property of plant based foods.

Waters *et al*., (2015) tried to prove the efficacy of prebiotics, probiotics and synbiotics in Irritable Bowel Syndrome (I.B.S.) and Chronic Idiopatic Constipation(C.I.C.) and concluded that prebiotics and probiotics symbiotically are beneficial for I.B.S. and C.I.C.

Rathore *et al*., (2012) tried to signify the role of prebiotics and probiotics on human health and found out that the application of probiotics done for treatment of diseases including viral, bacterial or antibiotic associated diarrhoea, lowering of serum cholesterol, decreased risk of colon cancer, improved lactose digestion, and altered intestinal microbiota. They also suggested the role of prebiotics in dietary intervention in colon cancer, acute infections and inflammatory bowel disease.

Martins *et al*., (2012) demonstrated *in vitro* and *in vivo* that probiotic therapies could be useful as adjuvant when treating gastrointestinal diseases. *S. boulardii, S. cerevisiae* and *S. cerevisiae BY474* strains were shown to capture certain pathogenic bacteria on their surface, preventing bacterial adhesion to specific receptors on the intestinal epithelium and subsequent invasion of the host.

Das *et al.,* (2012) studied the effects of probiotics on gut microbiota and found out that probiotics restore the composition of the gut microbiome and introduce beneficial functions to gut microbial or systemic disease phenotypes and they also concluded that diet and intestinal luminal conversion by gut microbes play a role in shaping the structure and function of intestinal microbial communities.

2.5 SCOPE AND FUTURE CHALLENGES

Technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way. This could make them ideal substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population. Probiotics products available in the markets today are mainly milk based; however, the increase in consumer vegetarianism and demand for cholesterol free probiotics has encouraged scientists and researchers to explore newer matrices as vehicles for probiotics particularly vegetable and fruit juices. Legumes contain beneficial nutrients, such as minerals, vitamins, dietary fibers, and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population, thus making them ideal substrates for probiotic cultures (Vasiljevic and Shah, 2008).

Patel & Goyal (2012) studied the current trend and future perspectives of prebiotics research and analysed the prebiotics production from new novel sources, from food industrial wastes, prebiotic supplementation in food, commercially available prebiotic agents, prebiotic production of various techniques and future perspectives.

The applicability of probiotics in food products depends in general on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH, mechanical stress, salt content, and content of other harmful or essential ingredients. For many products, excess water activity is a critical parameter that increases the death rate of bacteria. Products with an unfavourable water activity are, for example, cereals, chocolate, marmalade, honey, and toffees. These products are too “dry” for applying live bacteria and too “wet” for the application of freeze-dried bacteria. Freeze-dried bacteria could be applied in these products if the bacteria could be protected from moisture, as small amounts of moisture can be very detrimental to the dried culture. In addition to dairy products, fruit juices have been shown to be suitable carriers for probiotics. The limiting factor for many of the probiotic strains is the low pH of the juices. There is growing interest in consumers towards healthier foods and probiotic fruit, vegetable juices and cereal based beverages can serve as a good option. But, the application of probiotic cultures in non-dairy products and environments represents a great challenge and needs to be researched at the industrial level for commercial production of these healthy products (Vasiljevic and Shah, 2008).

3. SIGNIFICANCE OF THE STUDY

A healthy food means functional foods which exert beneficial effect on more specific body functions, in addition to the traditional nutritional effects. The well-known examples of functional foods are those containing or prepared with bioactive compounds like phytochemicals, dietary fibre, oligosaccharides and active friendly bacteria (probiotics) (Jankovic *et al*., 2010).

Within the functional foods, probiotics is the rapidly expanding active arena. Some researchers reported that a probiotic food represents 60-70% of functional foods (Tripathi and Giri, 2014). Beverages are by far the most active functional foods category because of convenience and possibility to meet consumer demands for container contents, size, shape, appearance, ease of distribution, better storage for refrigerated, shelf stable products and great opportunity to incorporate desirable nutrients and bioactive compounds (Kausar, 2012).

Indian probiotic industry is still in its infancy stage and presently accounts for only a small fraction (less than 1% of the total world market turnover in the probiotic industry). But probiotic industry is evolving at a steady pace with condition set for tremendous growth in near future. India is emerging as a major probiotic market of the future with annual growth rate of 22.6%. Until 2015 with a handful of players in Indian probiotic industry were Amul, Mother Dairy, Yakult, Danone and Nestle along with other minor players operating in different regions in their own capacities (Kausar, 2012).

A bacterial strain is not considered as a probiotic until the health benefits are clinically proven (Reid, 2008). Certain probiotic bacterial species, such as lactic acid bacteria, can adhere to the intestinal epithelium and thereby prevent invasion by pathogenic bacteria such as *Escherichia coli, Salmonella and Clostridium spp.* in the gut epithelium and this phenomenon is known as competitive exclusion. Feeding probiotics may also help to modulate the cellular and humoral immune system thereby enhancing the host's resistance to enteric pathogens (Vasiljevic and Shah, 2008).

The health effects attributed to probiotic include alleviation of lactose intolerance symptoms, treatment of viral and antibiotic associated diarrhoea, reduction of symptoms of antibiotic treatment of *Helicobacter pylori*, alleviation of atopic dermatitis symptoms in children, and prevention of the risk of allergy in infancy, alleviation of symptoms of inflammable bowel

disease (IBD) and irritable bowel syndrome (IBS) and enhancing the immune response (Saarela, 2000).

Although there are many benefits of having probiotics with dairy foods, non-dairy probiotic foods also play a significant role in human health. For instance, there are major drawbacks related to dairy foods such as allergy, lactose intolerance and cholesterol content, hence non-dairy probiotic foods are beneficial for the people having such health disorders. Furthermore, cultural (strict vegans) as well as specific religious believes among certain communities may also limit the consumption of dairy foods.

In such situations, non-dairy probiotic carrier foods and convenient mode of deliveries such as tablets could be the only way of providing probiotics. Each probiotic strain is unique in many aspects such as optimum growth conditions and growth medium. Consequently, the selection of probiotics which perform better or equally in non-dairy foods compared with dairy foods may be useful in developing non-dairy probiotic food (Gawkowski & Chikindas, 2013).

Supplementation of a probiotic-fermented drinks with the fructo-oligosaccharide (FOS), inulin and pectin increased the angiotensin I-converting enzyme inhibitory-activity and enhances antihypertensive effect (Yeo and Liong 2010).

Looking at the ever increasing demand of prebiotics recent trends in prebiotic production from new novel sources, prebiotic supplementation of food, prebiotic production by various techniques and future perspectives have been targeted in the present study.

Symbiotic effect makes it all the more beneficial and thus, legumes milk can be a source of probiotics and used to make it nutritionally adequate.

So the present study is planned to develop probiotic and prebiotic formulations commonly consumed by Indian population, beneficial for lactose-intolerant people, low in cholesterol and high in protein, cost effective and easily available.

4. OBJECTIVES

Keeping in view the nutritional and therapeutic importance of probiotics and prebiotics, the present study will be planned with the major objectives as follows:

- To develop legumes milk from (**Chickpea** (*Cicer arietinum*) & **Cowpea** (*Vigna unguiculata*) by different processing methods.

- To formulate non-dairy probiotic drink from legumes' milk.
- To enrich the non-dairy probiotic drink by adding prebiotics.
- To evaluate the organoleptic characteristics of the developed drink.
- To analyse the proximate composition of highly acceptable drink.
- To analyse the microbial load of the highly acceptable drink.
- To do the shelf life analysis of the highly acceptable drink.

5. RESEARCH DESIGN

PROCUREMENT OF LEGUMES [**Chick pea** (*Cicer arietinum*)- CSJK 114 & **Cowpea** (*Vigna unguiculata*)- RC-101 or RC 19] FROM R.S.S.C., DEPOT, DAGA SEEDS NEAR DURGAPURA AGRICULTURE INSTITUTE, JAIPUR.

METHODS USED FOR PROCESSING OF LEGUMES

- SOAKING : 12 HOURS.
- BLANCHING (80–100°C for less than 5 min) THEN SOAKING (12 hours)
- SOAKING AND THEN BLANCHING
- GERMINATION: Soaking for 4-5 hours and germination for 2 days.

EXTRACTION OF LEGUME MILK - The excess water after processing will be drained and then, the legumes will be wet-milled continuously for 5 min by using a homogenizer and filtered through double-layered cheesecloth. The filtrate of legume milk will be sterilized at 108 °C for 15 minutes.

SENSORY EVALUATION (for highly acceptable legume milk)

PROCUREMENT OF PROBIOTIC CULTURES

The cultures of *lactobacillus bulgaricus and lactobacillus bifidobacterium* will be obtained from the Institute Of Microbial Technology, Chandigarh.

ANALYSIS OF THE CRUDE FAT CONTENT OF THE HIGHLY ACCEPTABLE LEGUME MILK (to maintain the standards)

Open the glass ampoule by scoring the glass just above the cotton plug with a glass cutter or sharp file and breaking it at the scored mark. Pour the content of the ampoule into a liquid medium, or to a tube containing a solid agar slant with 2-3 drops of sterile water. After incubation the culture can be used as inoculum for plate cultures

BLENDING WITH SKIMMED MILK POWDER IN DIFFERENT PROPORTIONS (10%, 20%, 30%, 40% & 50%)

DEVELOPMENT OF PROBIOTIC FROM LEGUME MILK - Fermentation will be started by inoculation of bacterial cultures to achieve an initial count of 10^7 cfu/mL, followed by incubation at 37°C for 4-8 h. The fermented legume milk will be stored at 4°C.

ENRICHMENT WITH PREBIOTICS

- CHICORY ROOT POWDER (2%, 4%, 6%, 8% and 10%)
- RAW BANANA POWDER AND ALMONDS [(Banana-10:85, 20:75, 30:65, 40:55 & 50:45) & (Almonds- 5 grams)]

SELECTION OF PANEL MEMBERS (8-10) BY SENSITIVITY THRESHOLD TEST

ADDITION OF SUGAR (5%)

TRAINING OF THE SELECTED PANELLISTS FOR-

- Tasting of the probiotics available in the market (Yakult)
- Triangle test

ORGANOLEPTIC EVALUTION OF ALL THE VARIATIONS OF THE DEVELOPED DRINK ON HEDONIC RATING TEST

PROXIMATE ANALYSIS OF THE HIGHLY ACCEPTABLE PROBIOTIC DRINKS

ASH, MOISTURE, CRUDE FAT, CRUDE FIBER, CRUDE PROTEIN , CARBOHYDRATES AND ENERGY

pH BY pH-meter

ANTIOXIDANT ACTIVITY WILL BE ASSESSED BY D.P.P.H. ASSAY

SHELF LIFE STUDY (STANDARD PLATE COUNT) AT AN INTERVAL OF 5 DAYS OVER A PERIOD OF 30 DAYS AT A STORAGE TEMPERATURE OF 4°C

ASEPTIC BOTTLING AND STORING AT 4°C

MICROBIAL ANALYSIS FOR-

- the identification of specific bacteria.
- the total microbial load in the final drink.

STATISTICAL ANALYSIS

The results obtained will be subjected to analysis of variance (ANOVA). Mean comparison will be carried out among the 4 yogurt-like products by Statistical Programs for Social Sciences (S.P.S.S., 2011).

6. MATERIALS AND METHODS

6.1 METHODOLOGY

PHASE 1	Procurement of raw materials and processing of legumes.
PHASE 2	Extraction Of Legumes Milk and sensory evaluation of highly acceptable legume milk. And, development Of Probiotic drink From Legume Milk.
PHASE 3	Supplementation of probiotic drink With Prebiotics.
PHASE 4	Organoleptic evaluation, proximate analysis and microbial analysis of the developed beverage.
PHASE 5	Statistical analysis.

6.1.1 PHASE 1 - PROCUREMENT OF RAW MATERIALS

In the present research two legumes will be used for the preparation of non-dairy fermented drink. The two legumes selected are - **Chick pea** (*Cicer arietinum*) **&** **Cowpea** (*Vigna unguiculata*) which will be procured from Durgapura Agriculture Institute, Jaipur. The Chickpea variety CSJK-114 and the Cowpea variety RC-101 or RC-19 will be purchased from R.S.S.C. Depot, Daga Seeds, Durgapura.

The procured legumes will be visually inspected to remove damaged, deformed seeds, and any foreign material or stones. The cleaned legumes will be stored in food grade, air-tight plastic containers at room temperature.

PROCESSING OF LEGUMES

The clean, stored legumes will be hand-washed twice with potable water and then, will be subjected to various processing methods for the further extraction of legumes' milk.

- *SOAKING* : Soaking the legumes for 12 hours in distilled water with the addition of Citric acid 0.75 % w/v . The citric acid will be added for the purpose of seed coat softening (Buckle & Sambudi, 2013).
- *BLANCHING AND THEN SOAKING* : Aliquots (300 g) of dry seeds will be blanched in 2000 ml of distilled water for 5 minutes at 80-100℃. The blanched seeds will be drained and then placed in 1500 ml of distilled water and left to soak at 23 ± 2 ℃ for 12 h. (Buckle & Sambudi, 2013).

- *SOAKING AND THEN BLANCHING*: Soaking the legumes for 12 hours in distilled water at room temperature, followed by blanching for 5 minutes at 80-100^{o}C.

* In all the above mentioned processes, soaking water will be discarded as it is an effective way to reduce phytates and phytic acid from the legumes (Vadivel & Pugalenthi, 2008).

** Legume milk has a characteristic beany or nutty flavour, which is mainly due to lipo-peroxidation of polyunsaturated fatty acids (PUFA) mediated by lipoxygenases isoenzyme activity. These off- flavors could not be completely eliminated but minimized to a greater extent by heat treatment, alkali treatment or cleanliness. The use of a hot water (blanching) helps to remove its beany flavour (Kumar *et al*., 2002).

- *GERMINATION*: Two hundred g of legumes will be soaked in 1000 mL of 0.07% sodium hypochlorite (surface sterilization) for 30 minutes followed by washing and then, soaking in distilled water. These seeds will be introduced in the germination machine LCDS No 2555. The seeds will be germinated at 20°C, 99% relative humidity in darkness for 2 days. The maximum time of germination was fixed in accordance with achieving 95% sprout legume. The legumes will be germinated till the sprouts attain the size of ¼ to ½ inch (Uwaegbute *et al*., 2000).

6.1.2 PHASE 2 - EXTRACTION OF LEGUMES MILK

The excess water after processing will be drained and then, the legumes will be wet-milled continuously for 5 min by using a homogenizer and filtered through double-layered cheesecloth. The filtrate of legume milk will be sterilized at 108 ˚C for 15 minutes (Kale *et al*., 2012).

The analysis of the crude fat content of the obtained legume milk will be done for maintaining the standards of milk. The sensory evaluation will be done for the highly acceptable legume milk and the further addition of probiotics will be incorporated into the 100% legume milk and the legume milk blended with skimmed milk powder in different proportions as 10%, 20%, 30%, 40% & 50%.

Chien and Snyder (1983) reported that legume milk tasted less astringent by the addition of skimmed cow's milk, $CaSO_4$, or citric acid. Then stabilizers pectin or sodium alginates will be used at the rate of 0.4% and 0.6% w/w respectively (Kumar & Mishra, 2004).

6.1.3 PHASE 3 - DEVELOPMENT OF PROBIOTIC FROM LEGUME MILK

For the enhancement and maintenance of well-balanced intestinal microbiota, the legume milk will be fermented by live microbial cultures of *lactobacillus bulgaricus* and *bifidobacterium* to develop non-dairy fermented drinks.

In the present study, cultures of *lactobacillus bulgaricus* and *bifidobacterium* will be obtained from the Institute Of Microbial Technology, Chandigarh. These genera are selected because these are categorised under Generally-Recognized-As-Safe (GRAS) status, which indicates no or less health risks to the host upon consumption (Randheera *et al.,* 2010).

A freeze dried culture will be received in a glass ampoule containing a dried pellet with the microbe. The outer surface of the ampoule will be disinfected by cleaning it with 70% ethanol. Then, it is allowed to dry. Later, the ampoule was tapped gently on the work bench to loosen the frieze dried material from the glass. Open the glass ampoule by scoring the glass just above the cotton plug with a glass cutter or sharp file and breaking it at the scored mark. Later, the content of the ampoule was poured into a liquid medium, or to a tube containing a solid agar slant with 2-3 drops of sterile water. After incubation the culture will be used as inoculum for plate cultures. The inoculum level ranging from 6 - 10% and the incubation time varying from 4 to 6 hours will be used to standardise the probiotic drink. Inocula (1%) consisting of 24-h cultures grown in MRS medium will be used to initiate the fermentation. All inoculated extracts will be incubated at 37°C for 4-8 hours (Chou & Hou, 2000).

ENRICHMENT WITH PREBIOTICS

The obtained non-dairy probiotic drink will be supplemented with chicory root powder and the blend of banana & almonds (which will be procured from the market) which will be added in different proportions as mentioned below-

- *CHICORY ROOT POWDER* will be added in the proportion of 2%, 4%, 6%, 8% and 10% and the variations will be homogenized at 8000 rpm for 2 min in homogenizer.
- *RAW BANANA POWDER AND ALMONDS* will be incorporated in the proportions of 10:85, 20:75, 30:65, 40:55 & 50:45 and given numbers as variation 1, variation 2, variation 3, variation 4 & variation 5. The different variation will be homogenized at 8000 rpm for 2 min in homogenizer, before the addition of almonds in the standard amount of 5gms per variation.

After enrichment with prebiotics, two variations will be made, one with addition os sugar at 5% per variation and the other without sugar.

6.1.4 PHASE 4 - ORGANOLEPTIC EVALUTION OF THE DEVELOPED PRODUCT

(a) SELECTION OF PANELLISTS

The obtained samples will be subjected to sensory assessment by 8-10 panellists selected by the sensitivity-threshold test.

The selected panellists will be made familiar with the quality attributes of probiotic drinks available in the market (Yakult), and also they will be trained for Triangle test. Clean cups will be provided for each sample; each panellist will be requested to taste the samples one after the other and to indicate their degree of likeness or preference.

The triangle test is a discriminative method with many uses in sensory science including:

- gauging if an overall difference is present between two products
- selecting qualified panellists for a particular test
- determining whether shifts in processing or ingredients have significantly changed a product.

Test Principle

During a triangle test, a panellist is presented with one different and two a like samples. If possible, all three samples should be presented to the panellist at once, and the panellist should be instructed to taste the samples from left to right. The six possible order combinations should be randomized across panellists. For samples A and B, the six possible order combinations are: AAB, ABA, BAA, BBA, BAB, and ABB. The panellist is instructed to identify the odd sample and record his answer. (KABonsu *et al*., 2016; Srilakshmi, 2016).

(b) TEST USED FOR SENSORY EVALUATION

All the variations of developed non-dairy probiotic drink will be evaluated for organoleptic characteristics on Hedonic Rating Test on a 9 point scale ranging from 1.0 – 9.0 as Like Extremely, Like Very Much, Like Moderately, Like Slightly, Neither Like nor Dislike, Dislike Slightly , Dislike Moderately, Dislike Very Much and Dislike Extremely (Munoz & King, 2017; Srilakshmi, 2016).

All the developed products will be evaluated against standard probiotic drink (Yakult). Yakult *(Yakuruto*) is a probiotic dairy product made by fermenting a mixture of skimmed milk with a special strain of the bacterium *Lactobacillus casei Shirota*. Standard Yakult contains 14g of sugar for every 100g, but comes in 65 ml bottles with the shelf life of 21 days under refrigeration (4℃).

PROXIMATE ANALYSIS OF THE DEVELOPED BEVERAGE

After the organoleptic evaluation, the beverage samples were stored in refrigerator at 4℃ and will be analyzed for pH and %Acidity. And the proximate analysis will be carried out for the highly acceptable non-dairy fermented drink according to the standard protocol A.O.A.C., (2016).

S. No.	NUTRIENT	METHOD	REFERENCE
1.	MOISTURE (g/100g)	Oven drying method	A.O.A.C., (2016)
2.	ASH (g/100g)	Dry ashing method	A.O.A.C., (2016)
3.	CRUDE FIBRE (g/100g)	Acid Alkali Wash method	A.O.A.C., (2016)
4.	FAT (g/100g)	Ether Soxhlet Extract method	A.O.A.C., (2016)
5.	PROTEIN (g/100g)	Micro Kjeldahl method	A.O.A.C., (2016)
6.	CARBOHYDRATES	Composite method	A.O.A.C., (2016)
7.	TOTAL ENERGY (Kcal/100g)	By Calculation	

ANALYSIS OF ANTIOXIDANT ACTIVITY

METHOD	REFERENCE
DPPH (**1,1-diphenyl-2-picrylhydrazyl) assay**	Sonia *et al*., (2016)

CELL VIABILITY

It is necessary that the probiotic culture contain sufficient number of viable microbial cells. The probiotic cell viability dramatically decreases because of exposure to detrimental environmental factors such as organic acids, hydrogen ions, molecular oxygen and antibacterial components (Shah and Lankaputhra, 1997; Sultana et al., 2000). The beneficial effects of probiotic microorganisms appear when they arrive in the intestinal medium, viable and in high enough number, after surviving the above mentioned harsh conditions (Gilliland, 1989). According to the International Dairy Federation (IDF) recommendation, this index should be $\geq 10^7$ CFU/g up to the date of minimum durability (Ouwehand and Salminen, 1998).

SHELF LIFE STUDY

The microbial analysis of the final aseptic bottled beverage will be done for the identification of the specific bacteria and to assess the total microbial load in the final drink.

The Standard Plate Count (S.P.C.) of the prepared drink will be done at an interval of 5 days over a period of 30 days at a storage temperature of 4°C (Cappuccino & Sherman, 2016, Rathore *et al.*, 2012).

METHOD	REFERENCE
SPC (Standard Plate Count)	Cappuccino & Sherman (2014)

YEAST AND MOLD COUNT

Microbial analysis will be done to determine total yeast and mold count of the probiotic beverage on the potato dextrose agar media for yeast and mold count by the method recommended by Harrigan and Mc.Cance, (1966). Pour plate technique will be used to determine yeast and mold count. The PDA media will be prepared and sterilized at pressure (15 psi) for 15min. The dilutions of sample will be made up to 8 dilutions and 1 mL of aliquot was used for plating. Plates will be incubated at 37°C for 48-72h, and results noted in CFU/mL. The yeast and mold count of the drink will be checked at weekly intervals for 4 weeks.

COLIFORM TEST

The Coliform and basically *E.Coli* are the indicator microbes of water contamination by faeces and therefore it is mandatory to examine the contamination. The Coliform gives red pink colonies on VRB agar, therefore it will be used for examination. Using the pour-plate technique, appropriately 1 mL aliquots will be taken in duplicate plates and tempered VRB agar will be added. The agar will be allowed to solidify and then another layer of about 5 mL of VRB agar will be added and allowed to solidify again. Plates will be inverted and incubated at 35℃ for 24 h. Red colonies surrounded by a zone of precipitate and report as presumptive coliforms in CFU/mL.

6.1.5 PHASE 5- STATISTICAL ANALYSIS

The results obtained were subjected to analysis of variance (ANOVA). (Bryman & Cramer, 2011). Mean comparison were carried out among the 4 yogurt-like products by Statistical Programs for Social Sciences (S.P.S.S., 2011).

7. FACILITIES AVAILABLE AT THE PLACE OF WORK

S.NO.	NAME OF EQUIPMENT	MAKE
1.	Refrigerator Double Door	LG
2.	Gas burner 4 gas	FABER
3.	Hand Blender	INALSA
4.	Food Weighing machine	RAJASTHAN SURGICAL SUPPLY
5.	Spectrophotometer	SYNTRONICS
6.	KEL PLUS (Nitrogen Digestion System)	Model KE: 04L (Digestion unit) Model: Elite GC Distillation
7.	SOCS PLUS	Model: SCS-4 (Pat Echoector)
8.	FIBRA PLUS	Model: FES-02
9.	Physical Balance	JEEVAN NAGJI & CO.
10.	Moisture Analyzer	MODELS : TMA-TOSHNIWAL AND CO.07.
11.	Digital Electronic Balance	SARTORIOUS GERMANY , CAT NO. BSA224
12.	Hot Air Oven	MAC MODEL OVD95 (SAD)
13.	Muffle Furnace	MAC MODEL METI-12
14.	Centrifuge Machine	REMI MODEL R-8C
15.	Seed Germinator Plus	LCDS No 2555

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APPENDIX

SPECIMEN EVALUATION CARD FOR TRIANGLE TEST

NAME :……. DATE :……

PRODUCT :…….....

Two Of The Three Samples Are Identical. Determine The Odd Sample.

SET NO.	CODE NO. OF SAMPLES	CODE NO. OF ODD SAMPLE	COMMENT ON ODD SAMPLES
1			
2			
3			
4			
5			

Signature……..

(Srilakshmi, 2016)

SPECIMEN EVALUATION CARD FOR HEDONIC RATING TEST

NAME :…… DATE :…….

PRODUCT :……

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feelings about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. As honest expression of your personal feelings will help us.

	CODE	CODE	CODE
Like Extremely			
Like Very Much			
Like Moderately			
Like Slightly			
Neither Like Nor Dislike			
Dislike Slightly			
Dislike Moderately			
Dislike Very Much			
Dislike Extremely			
REASON			

Signature :…….

(Srilakshmi, 2016)